

PCTWORLD INTELLECTUAL
PROPERTY ORGANIZATION

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)



WO 9602245A1

(51) International Patent Classification 6 :

A61K 31/195, 31/00

A1

(43) International Publication Date: 1 February 1996 (01.02.96)

(21) International Application Number: PCT/GB95/01657

(22) International Filing Date: 13 July 1995 (13.07.95)

(30) Priority Data:

275,167

14 July 1994 (14.07.94)

US

(71) Applicant (for all designated States except US): THE WELL-
COME FOUNDATION LIMITED [GB/GB]; Unicom
House, 160 Euston Road, London NW1 2BP (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): ZIMMERMAN, Thomas,
Paul [US/US]; 6209 Braes Meadow, Raleigh, NC 27612
(US). MOLINA, Luis [US/US]; 2135 North Lakeshore
Drive, Chapel Hill, NC 27514 (US).(74) Agent: HAMMER, Catriona, McLeod; The Wellcome Foun-
dation Limited, Glaxo Wellcome House, Berkeley Avenue,
Greenford, Middlesex UB6 0NN (GB).(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH,
CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE,
KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN,
MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE,
CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT,
SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML,
MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ,
UG).

Published

With international search report.

(54) Title: NITRIC OXIDE SYNTHASE INHIBITORS FOR INHIBITING THE PRODUCTION OF AIRWAY MUCUS

(57) Abstract

The use of an NO synthase inhibitor, for example L-NMMA, in inhibiting the production of airway mucus secretion, e.g. in cystic fibrosis, chronic bronchitis and emphysema, is disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

NITRIC OXIDE SYNTHASE INHIBITORS FOR INHIBITING THE PRODUCTION OF AIRWAY MUCUS

The present invention relates to the use of nitric oxide synthase inhibitors in inhibiting secretion and/or production of airway mucus, in particular in treating chronic bronchitis, cystic fibrosis and bronchorrhea.

In cystic fibrosis several functions of airway epithelia are abnormal including thickened airway surface fluid. This thickened airway fluid contributes to the recurrent pulmonary infections and loss of ventilatory function that occur in cystic fibrosis. Retained airway secretions are also known to contribute to the morbidity of other pulmonary diseases such as asthma and chronic obstructive pulmonary disease.

The therapeutic goal in cystic fibrosis and other pulmonary diseases in which the water content of the mucus is altered is to remove retained secretions from the lungs. For example, the use of aerosolised amiloride to facilitate the removal of retained mucus secretions is described in U.S. Patent No. 4,501,729. Amiloride appears to block Na^+ reabsorption by airway epithelial cells, and therefore inhibits water absorption from mucus.

A different therapeutic approach is to increase the water content of the airway surface liquid by modulating the activity of chloride channels. An example of this is the administration of ATP or UTP, which appear to induce hydrated mucus secretions by stimulating chloride secretion from respiratory epithelial cells. See, e.g., C.Stock, *Breathing Easier: A promising Treatment for Cystic Fibrosis*, *Endeavors*, 10, 10-11 (Fall 1992) (Published by the Office of Research Services, The University of North Carolina at Chapel Hill).

It has subsequently been found that administering a nitric oxide synthase inhibitor is beneficial in inhibiting the production and/or secretion of airway mucus.

Accordingly, the present invention provides a method of inhibiting the production and/or secretion of airway mucus which comprises administering to a mammal in need thereof an effective amount of an NO synthase inhibitor to the lungs of the mammal.

In a further aspect, the present invention provides a method of combating cystic fibrosis

-2-

which comprises administering to a mammal in need thereof an effective amount of an NO synthase inhibitor to the mammal.

In a yet further aspect, the present invention provides a method of combating excessive mucus production in chronic bronchitis which comprises administering to a mammal in need thereof an effective amount of an NO synthase inhibitor to the mammal.

In a still further aspect the present invention provides a method of combating emphysema which comprises administering to a mammal in need thereof an effective amount of an NO synthase inhibitor to the mammal.

The method of the present invention may further comprise the step of concurrently administering a further active agent useful in improving mucociliary clearance of airway mucus, for example a sodium channel blocker, such as amiloride, in an amount effective to inhibit the reabsorption of water from lung mucus secretions, or a lantibiotic such as duramycin, or a nucleotide such as adenosine triphosphate (ATP) or uridine triphosphate (UTP) in an amount effective to facilitate hydration and clearance of airway mucus.

In the alternative there is provided the use of a NO synthase inhibitor in the manufacture of a medicament to facilitate mucus clearance

The present invention also provides the use of an NO synthase inhibitor for the manufacture of a medicament for the inhibition of the production of airway mucus in a patient in need of such treatment. Suitably the patient is suffering from cystic fibrosis or chronic bronchitis.

Suitably the NO synthase inhibitor is an arginine derivative such as those described in U.S. Patent 5028627 and preferably it is NG-monoethyl-L-arginine (L-NMMA) or a salt thereof.

L-NMMA is available from Sigma Chemical Company Limited, Fancy Road, Poole, Dorset BH17 7NH, England.

Many NO synthase inhibitors, for example L-NMMA, are capable of forming salts. Thus, the present invention includes NO synthase inhibitors in the form of salts, in

-3-

particular acid addition salts. Suitable salts include those formed with both organic and inorganic acids. Such acid addition salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable salts may be of utility in the preparation and purification of the compound in question. Thus, preferred salts include those formed from hydrochloric, hydrobromic, sulphuric, citric, tartaric, phosphoric, lactic, pyruvic, acetic, succinic, oxalic, fumaric, maleic, oxaloacetic, methanesulphonic, ethanesulphonic, p-toluenesulphonic, benzenesulphonic and isethionic acids. Salts of the compounds of NO-synthase inhibitors can be made by reacting the appropriate compound in the form of the free base with the appropriate acid.

Whilst it may be possible for the NO-synthase inhibitors to be administered as the raw chemical, it is preferable to present them as a pharmaceutical formulation. According to a further aspect, the present invention provides a pharmaceutical formulation comprising the NO synthase inhibitor or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), intranasal, inhaled, rectal and topical (including dermal, buccal, sublingual and intraocular) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the NO-synthase inhibitor or a pharmaceutically acceptable salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in

-4-

an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

Formulations for topical administration in the mouth, for example sublingually, include lozenges comprising the active ingredient in a flavoured basis such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a basis such as gelatin and glycerin or sucrose and acacia.

Formulations suitable for pulmonary administration via the buccal cavity are presented such that particles containing the active ingredient and desirably having a diameter in the range 0.5 to 7 microns are delivered into the bronchial tree of the recipient. Such

formulations may be in the form of finely comminuted powders which may conveniently be presented in a pierceable capsule, for example of gelatin, for use in an inhalation device, or as a self-propelling formulation (also referred to as an aerosol formulation) comprising the active ingredient a suitable liquid or gaseous propellant and optionally other ingredients such as a surfactant and/or a solid diluent. Suitable liquid propellants include propane and the chlorofluorocarbons, and suitable gaseous propellants include carbon dioxide. Suitable surfactants include sorbitan trioleate (which is available for example under the trade name "Arlacel 85"), Polysorbate 20 and oleic acid. Self-propelling formulations may also be employed wherein the active ingredient is dispensed in the form of droplets of solution or suspension. The self-propelling formulation typically contains from 0.05 to 20mg/ml e.g. 0.1 to 5mg/ml of the active ingredient.

Such self-propelling formulations are analogous to those known in the art and may be prepared by established procedures. Suitably they are presented in a container provided with either a manually-operable or automatically functioning valve having the desired spray characteristics; advantageously the valve is of a metered type delivering a fixed volume, for example, 25 to 100 microlitres, upon each operation thereof.

As a further possibility, the active ingredient may be in the form of a solution or suspension for use in an atomiser or nebuliser whereby an accelerated airstream or ultrasonic agitation is employed to produce a fine droplet mist for inhalation. Such solutions or suspensions may comprise, in addition to the naphthoquinone and solvent(s), optional ingredients such as surfactants. Suitable surfactants include those described above for self-propelling formulations. The solution or suspension typically contains from 500 μ M to 100mM e.g. 0.5mM to 25mM of the active ingredient. When a suspension of the active ingredient is employed, this compound is preferably in finely divided form, e.g. in micronised form.

Formulations suitable for nasal administration include presentations generally similar to those described above for pulmonary administration. When dispensed such formulations should desirably have a particle diameter in the range 10 to 200 microns to enable retention in the nasal cavity; this may be achieved by, as appropriate, use of a powder of a suitable particle size or choice of an appropriate valve. Other suitable formulations include coarse powders having a particle diameter in the range 20 to 500 microns, for administration by rapid inhalation through the nasal passage from a container held close

-6-

up to the nose, and nasal drops comprising 0.2 to 5% w/v of the active ingredient in aqueous or oily solution or suspension.

Preferred unit dosage formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

The NO synthase inhibitors of the invention may be administered orally, or via injection at a dose of from 1 to 100mg/kg per day. When the NO synthase inhibitors are given by injection, this will normally be in the form of an intravenous bolus or by infusion, preferably the latter. The dose range for adult humans is generally from 70mg to 2.5g/day and preferably 150mg to 2g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound of the invention which is effective at such dosage or as a multiple of the same, for instance, units containing 5mg to 500mg, usually around 10mg to 200mg.

In a further embodiment of the invention, the NO synthase inhibitors are administered by aerosol to the lungs of a subject in an amount sufficient to achieve concentrations of NO synthase inhibitor on the airway surface liquid of the patient of about 5 μ Moles/litre to 1000 μ Moles/litre, preferably 100 μ Moles/litre to 500 μ Moles/litre.

The dose of the NO synthase inhibitor will vary according to the potency of the inhibitor, its selectivity for the different forms of the enzyme and the dose at which adverse pharmacological effects become evident. The person skilled in the art will take these factors into account when determining the dose of inhibitor to be administered.

-7-

The present invention will now be described by way of example only.

Example

SPOC-1 Cell Line Synopsis

The experimental mode used is the SPOC-1 cell line, a mucin-secreting cell line that results from a spontaneous transformation in a primary culture of rat tracheal cells. SPOC-1 cells seeded in a rat tracheal xenograft and implanted into a nude mouse differentiate into a pseudo-stratified epithelium in which the upper layer of cells possesses a goblet cell phenotype, including the possession of $1 + \mu\text{m}$ granules. In culture either on plastic or on Transwell-Col (Costar) permeable supports, the cells differentiate over a period of 9 to 12 days into a multilayered (3 to 4 layers) epithelium in which outer layers contain material which stains positive with PAS. A PAS-positive glycoconjugate with a molecular weight appropriate for mucin ($>10^6$ daltons) is detected by gel electrophoresis of SPOC cell lysate and perfusate; and SPOC cells stain positive with an monoclonal antibody specific to purified rat mucin.

An enzyme-linked lectin binding assay (ELLA-is analogous to ELISA but uses a lectin instead of an antibody) that is sensitive to the ng/ml range and a perfusion system for cultures growing on both plastic (12-well plates) and TCol supports has been developed.

Testing Procedure

SPOC-1 cells grown on plastic are employed as a convenient experimental model to test chemical compounds for inhibitory or stimulatory effects on mucin secretion. Each compound is initially tested, on duplicate cultures in short-term exposures, for potential effects on both baseline and ATP-stimulated secretion over a range of 12 doses (including a control). 12-well plates are perfused (each well independently), and after a suitable equilibration period, 5 min fractions collected for baseline (30 min), baseline + test compound (60 min), and ATP + test compound (60 min). A level of ATP at its EC_{50} , $10\mu\text{M}$ is used, so potential stimulatory effects on regulated mucin secretion are detected with the compounds of interest; if the primary interest is in the inhibitory direction, then a maximal ATP dose, 100 or $300\mu\text{M}$ is used.

-8-

The fractions collected are assessed for mucin content by ELLA, and the data analysed as follows, using standard spreadsheet software (MS Excel). Data from duplicate samples is averaged. The time course of mucin secretion for each perfused culture is plotted, and the levels of mucin secretion assessed for each period (baseline, baseline + compound, and compound + ATP). Calculating the data relative to baseline rates, dose-response curves for the baseline + compound and the compound +ATP periods are constructed. These plots are analysed for inhibitory or stimulatory effects by the compounds of interest, by comparing data for each baseline + compound period to the preceding baseline, and for each compound + ATP period to the preceding baseline + compound period.

Scientists from North Carolina State University have examined the mechanism involved in stimulation of mucus secretion. Stimulation of mucus secretion was completely blocked by L-NMMA at a dose of 0.1-1.0mM, but not by D-NMMA, indicating the role of nitric oxide in mucus secretion (K.B.Adler et al., Chest, 107(3), 133S-135S, 1995).

CLAIMS

1. Use of an NO synthase inhibitor in the manufacture of a medicament for the inhibition of the production of airway mucus secretion in a mammal.
2. Use according to Claim 1 for combating cystic fibrosis in a mammal.
3. Use according to Claim 1 for combating excessive mucus production in chronic bronchitis in a mammal.
4. Use according to Claim 1 for combating emphysema in a mammal.
5. Use according to any one of Claims 1 to 4 wherein the NO synthase inhibitor is L-NMMA.
6. The use of a NO synthase inhibitor according to any one of Claims 1 to 6 in conjunction with a further active ingredient useful in improving mucociliary clearance of airway mucus.
7. A method of inhibiting the production of airway mucus in a patient in need of such treatment which comprises administering an effective amount of an NO synthase inhibitor to the lungs of the mammal.
8. A formulation comprising an NO synthase inhibitor together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients, said formulation being adapted for inhalation or intranasal administration.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 95/01657

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-5317040	31-05-94	NONE	
US-A-5028627	02-07-91	AU-B- 634826	04-03-93
		AU-B- 6409690	18-04-91
		CA-A- 2065040	14-03-91
		EP-A- 0597831	25-05-94
		JP-T- 5500219	21-01-93
		WO-A- 9104024	04-04-91
		US-A- 5216025	01-06-93
		US-A- 5312835	17-05-94

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB95/01657

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 7 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

National Application No
PCT/GB 95/01657

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J.COMP.NEUROL., vol. 345, no. 1, 1 July 1994 pages 125-38, 'Neural nitric oxide synthase is localized in extrinsic nerves regulating perireceptor processes in the chemosensory nasal mucosae of rats and humans' see page 133, left column, line 32 - right column, line 3 see page 135, left column, line 50 - line 55 see page 135, right column, line 5 - line 12 see page 136, last paragraph ---	1-7
X,P	CHEST, vol. 107, no. 3, 3 March 1995 pages 133S-135S, 'TNF-alpha stimulates mucin secretion and gene expression in airway epithelium in vitro' cited in the application see page 134S, right column, line 35 - line 39 ---	1-7
A	N.ENGL.J.MED., vol. 324, no. 22, 1991 pages 1539-47, 'Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease' see page 1540; table 1 see page 1541, left column, line 20 - line 22 see page 1542, right column, line 28 - page 1543, left column, line 3 see page 1544; table 3 see page 1544, right column, line 11 - line 28 ---	1-7
A	US,A,5 028 627 (KILBOURN ET AL.) 2 July 1991 cited in the application see the whole document -----	1-8

INTERNATIONAL SEARCH REPORT

National Application No
PCT/GB 95/01657

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/195 A61K31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 317 040 (GOLDMAN) 31 May 1994 see column 3, line 62 - column 4, line 5 see column 5, line 22 - column 6, line 5	8
Y	--- -/--	1-7

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "a" document member of the same patent family

Date of the actual completion of the international search

24 October 1995

Date of mailing of the international search report

17.11.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Gerli, P